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(54) Title: INTERNAL 1,15-LACTONES OF FLUPROSTENOL AND RELATED PROSTAGLANDIN $F_{2\alpha}$ ANALOGS AND THEIR USE IN THE TREATMENT OF GLAUCOMA AND INTRAOCULAR HYPERTENSION

(57) Abstract: Novel derivatives of prostaglandin compounds of the F-series (PGF), specifically macrocyclic internal 1,15-lactones of fluprostenol and related PGF analogs, such as cloprostenol or latanoprost. The novel analogs can be formulated into ophthalmic solutions and topically applied for the treatment of the increased intraocular pressure caused by glaucoma and the reduction of ocular hypertension.

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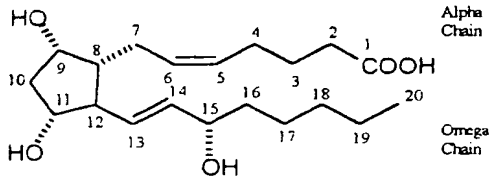
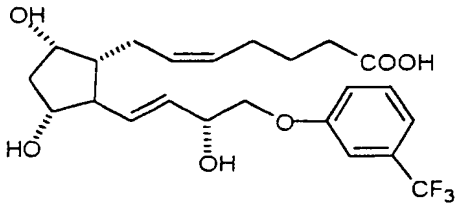
A number of simple $PGF_{2\alpha}$ analog esters have been found to be potent and selective agents useful for the treatment of ocular hypertension. For example, latanoprost is the isopropyl ester of 13,14-dihydro-17-phenyl-18,19,20-trinor $PGF_{2\alpha}$ and is widely marketed for the clinical treatment of glaucoma under the trade name Xalatan. See monograph 5387, page 918 of *The Merck Index*, 12th edition (1996). Likewise, the isopropyl ester of fluprostenol and of similar $PGF_{2\alpha}$ analogs, such as cloprostenol, are specifically claimed as ocular antihypertensive agents in U.S. patent 5,665,773. The structures of naturally-occurring $PGF_{2\alpha}$ (Structure I), fluprostenol (Structure II), and latanoprost (Structure III) are shown hereinbelow. For a review of these agents, see Lindén and Alm, *Drugs and Aging*, Vol.14, No. 5, pp. 387-398 (1999).



Structure I



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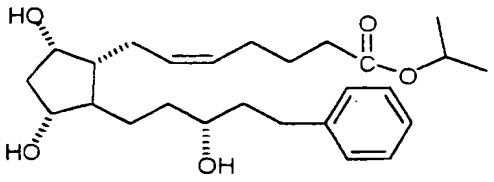


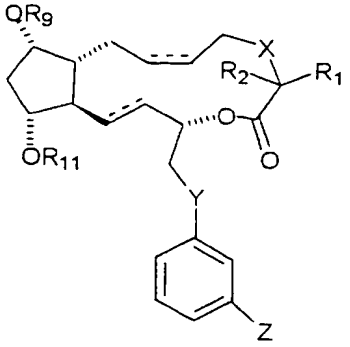
Structure III

prostaglandins, and in particular internal 1,15-lactones of $PGF_{2\alpha}$ analogs, such as the 16-aryloxy prostaglandin analogs, illustratively fluprostenol or cloprostenol.

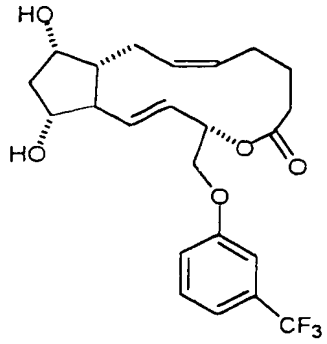
We have discovered that it is possible to form an internal ester of $PGF_{2\alpha}$ analogs by creating a carbon-oxygen bond between the alcohol group at C-15 and the C-1 carboxylic acid. This creates a macrocyclic lactone that has novel and desirable characteristics. In fact, some of the novel analogs form highly crystalline structures that are easy to formulate into ophthalmic solutions, for example. The hydrolysis of these $PGF_{2\alpha}$ analog 1,15-lactones releases only the active $PGF_{2\alpha}$ analog free acid, without the production of a small aliphatic alcohol coproduct. Thus, these compounds are ideal and unique prodrugs for the treatment of glaucoma and other disorders causing an increase in intraocular pressure in the eyes of humans or animals.

For the purposes of this patent, the term "prostaglandin" is intended to mean any one of the prostanoic acid derivatives which include the ring type A, B, C, D, E, F, G, H, I, J and K, but most particularly those of the F-type. The term "derivative" is intended to mean all compounds which have a chemical affinity, resemblance, or structural character which clearly associates them with the prostanoids and in particular, prostanoic acid or $PGF_{2\alpha}$. The term "analog" is intended to mean any somewhat modified version of a natural product, in this case a prostaglandin, or a related synthetic analog, wherein a number of atoms such as carbon, hydrogen, oxygen or heteroatoms such as nitrogen, sulfur or halide have been added or deleted from the parent structure, so as to yield a new molecular compound.

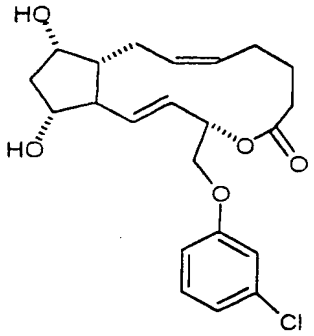
The compounds of the present invention have the general Formula I:



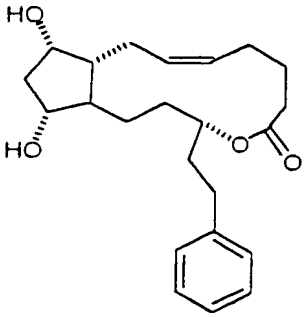
Formula I



Structure IV



Structure V



Structure VI

osmolarity adjusting agents, such as sodium chloride (NaCl) or potassium chloride (KCl), as is known in the art. Other ingredients which may be desirable to use in the ophthalmic preparations of the present invention include preservatives, co-solvents and viscosity building agents.

Ophthalmic products are typically packaged in multidose form, which generally require the addition of preservatives to prevent microbial contamination during use. Suitable preservatives include: benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, ONAMER M®, or other agents known to those skilled in the art. Such preservatives are typically employed at a concentration between about 0.001% and 1.0% by weight.

Prostaglandins, and particularly ester derivatives, typically have limited solubility in water and therefore may require a surfactant or other appropriate co-solvent in the composition. Such co-solvents include: Polysorbate 20, 60 and 80; Pluronic F-68, F-84 and P-103; Tyloxapol®; Cremophor® EL; sodium dodecyl sulfate; glycerol; PEG 400; propylene glycol; cyclodextrins; or other agents known to those skilled in the art. Such co-solvents are typically employed at a concentration between about 0.01% and about 2% by weight.

Viscosity greater than that of simple aqueous solutions may be desirable to increase ocular absorption of the active compound, to decrease variability in dispensing the formulations, to decrease physical separation of components of a suspension or emulsion of formulation and/or otherwise to improve the ophthalmic formulation. Such viscosity building agents include, for example, polyvinyl alcohol, polyvinyl pyrrolidone, methyl cellulose, hydroxy propyl methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxy propyl cellulose or other agents known to those skilled in the art. Such agents are typically employed at a concentration between about 0.01% and about 2% by weight.

In a specific illustrative embodiment, a topical formulation for treating increased intraocular pressure, in accordance with the invention, comprises:

A 4 L, 3-neck round-bottom flask was dried in an oven at 110°C overnight and fitted with an addition funnel, an overhead stirrer and a dry nitrogen gas inlet tube. Sodium hydride (NaH; 0.617 moles; 14.8 g) as a dry powder was suspended in 1.5 L of anhydrous tetrahydrofuran (THF). The mixture was cooled to 0°C with an external ice bath and 75 ml (100 g; 0.617 moles) of *m*-trifluoromethyl cresol was added dropwise and stirred one hour at 0°C and 2 hours at 22°C. The reaction mixture was then cooled to 0°C and (47.3 ml; 0.5 moles) of methyl bromoacetate was added dropwise. The mixture was stirred an additional 2 hours at 0°C and 1 hour at 22°C. 1.5 L of ethyl acetate was then added and the mixture transferred to a 6 L separatory funnel. 2 L of water was added and the layers were separated. The organic layer was washed twice with 1 L of brine, dried over solid anhydrous sodium sulfate, and the solvents evaporated to give 150 g of the trifluoromethyl compound ii as a yellow oil.

This product may be used directly in the next step of the synthesis, or more reliably may be purified by flash chromatography and used in a more purified form. The trifluoromethyl compound ii (86 g) is dissolved in 1.5 L of anhydrous THF and placed in a 2 L addition funnel over a 3-neck, 3 L round-bottom flask under dry nitrogen. Dimethyl methylphosphonate (63.3 ml) was added directly to the 3 L flask along with 1.2 L of anhydrous THF and cooled to -78°C with an external dry ice / acetone bath while stirring well with a mechanical stirrer. 2.5 M n-butyl lithium (217.6 ml) was added dropwise. The mixture was stirred at -78° for 90 minutes, and the solution of compound ii was then added dropwise over 30 minutes. The reaction was maintained an additional 4 hours at -78°C then stirred at ambient temperature overnight. The reaction mixture was then acidified with 2 L of 5% potassium hydrogen sulfate ($KHSO_4$) and transferred to a 6 L separatory funnel. It was diluted with 1.5 L of ethyl acetate, and the aqueous layer extracted once with 1 L of ethyl acetate and discarded. The organic layers were combined and washed with 1 L portions of brine until neutral, then dried over solid sodium sulfate and the solvent evaporated to give 237 g of yellow oil. This oil was purified by flash chromatography on silicic acid packed and eluted with 20:80 hexane:ethyl acetate. Pure fractions were combined and evaporated to give 175 g of the phosphonate compound iii.

sulfate. Removal of solvent on a rotary evaporator under reduced pressure afforded 200 g of an oil. The oil was purified on a silica gel column using 10:90 acetone:hexane as the eluant to afford 60 g of the benzoate alcohol compound v.

A solution of the benzoate alcohol compound v (60 g; 126 mmol) in 900 ml of methanol was placed in a 2 L round-bottom flask. Potassium carbonate (K_2CO_3; 21g; 159 mmol) was added and the reaction mixture was stirred at ambient temperature for 90 minutes. The reaction mixture was cooled to 0°C and acidified to pH 6 with 5% potassium hydrogen sulfate. The reaction mixture was diluted with 1500 ml of brine and extracted twice with 1 L of ethyl acetate. The organic layers were combined and washed with brine until it had a neutral pH. The organic phase was dried over sodium sulfate and concentrated on a rotary evaporator under reduced pressure to afford an oil which was purified on a silica gel column using 90:10 ethyl acetate:hexane as the eluant to furnish the desired lactone diol compound vi.

A 3 L jacketed-flask was equipped with a mechanical stirrer and a temperature microprocessor. The flask was charged with the lactone diol compound vi (~148 g; 0.397 moles) and approximately 2000 ml of methylene chloride under an atmosphere of nitrogen. This mixture was stirred until dissolved.

Approximately 7 equivalents of ethyl vinyl ether (266 ml; 2.779 moles) was added to the flask followed by the addition of approximately 0.1 equivalents of trichloroacetic acid (6.49 g; 0.0397 moles). The reaction mixture was stirred at room temperature until the reaction was judged to be complete by monitoring the reaction progress with thin layer chromatography (TLC). In this case, the reaction mixture was spotted on a silica gel TLC plate alongside a spot of the starting material. The spotted plate was placed into a TLC tank containing 80% ethyl acetate, 20% hexane (v/v). To develop, the TLC plate was sprayed with a 50:50 mixture of sulfuric acid and water (v/v) and heated. In some instances, it may be necessary to heat the reaction mixture to 30°C ± 5° for the reaction to go to completion.

While the reaction mixture is going to completion, a 10% potassium bicarbonate solution was prepared by combining approximately 10 g of potassium bicarbonate with approximately 100 ml of tap water in a 250 ml Erlenmeyer flask and swirling until

was spotted on a silica gel TLC plate alongside a spot of the starting material. The spotted plate was placed into a TLC tank containing 50% ethyl acetate, 50% hexane (v/v). To develop, the TLC plate was sprayed with a 50:50 mixture of sulfuric acid and water (v/v) and charred with heat. Once the reaction was judged to be complete, the heating circulator was turned off.

Approximately 0.31 equivalents of tap water (to DIBAL) and approximately 700 ml THF was combined in a 1 L Erlenmeyer flask and chilled. Excess DIBAL was decomposed by adding the chilled mixture of water and THF to the stirring solution through the addition funnel attached to the 6 L reaction vessel. The water/THF solution should be added dropwise and slowly. In particular, the first 50-100 ml should be added very slowly because foaming can occur. The temperature was allowed to rise during the addition. Once the addition is complete, the temperature should be between 0°C to -45°C.

Using the Fisher circulator, the reaction was warmed to approximately 20°C and then stirred for about 1 hour. A temperature of about 28°C ±10°C should be maintained. After about 30 minutes, the reaction tends to heat up because the salts are hydrating. Over the 1 hour period, the reaction mixture went from a dull yellowish-brown color to a titanium white slurry.

Approximately 990 ml toluene and approximately 660 ml THF was combined in a separate flask. Approximately 2 inches of celite 545 was placed in a 2 L fritted-funnel and enough of the mixture was poured over the top of the celite so that it was totally covered. Once the reaction was complete, the slurry was filtered over the celite using a water aspirator for suction. A stream of nitrogen was aimed at the filter funnel during filtration. The filter cake and reaction vessel was washed with the toluene/THF. The filter cake was discarded. The solvent was evaporated to give lactol compound viii as a viscous yellow oil which was used directly, without purification in the next step.

4-Carboxybutyl triphenylphosphine bromide (8.57 g; 19.34) was suspended in 30 ml of THF (anhydrous). Potassium *tert*-butoxide (38.68 ml; 38.68 mmol) was slowly added to this suspension. The reaction mixture was stirred at room temperature for 45 minutes and then cooled down to -10°C with ice/NaCl. Subsequently, lactol compound

sulfate and evaporated. The residue was purified on an acid-washed silica gel column using 10:90 ethyl acetate/hexane as eluent. Mass of collected product, 9-TBDMS-fluprostenol 11,15-diethoxyethyl ether, compound xii, was 5.64 (95.40% yield).

Compound xii (3 g) was dissolved in 100 ml of THF in a 500 ml round-bottom flask and stirred, under a nitrogen atmosphere, at room temperature. Following the addition of 0.5 M hydrochloric acid (2.0 ml), the reaction mixture was stirred at ambient temperature for 2 hours. The reaction mixture was then diluted with ethyl acetate, saturated with brine, and extracted once with ethyl acetate. The combined organic solvents were dried over anhydrous sodium sulfate and the solvents were removed under reduced pressure to give 2.57 grams of fluprostenol 9-TBDMS ether, compound xiii, as a viscous oil.

Compound xiii (2.57 g; 5.9 mmol) was dissolved in 30 ml of anhydrous (oxygen free) xylene. To this solution, 2,2'-dipyridyl bisulfide (1.59 g; 7.2 mmol) and triphenylphosphine (1.89 g; 7.2 mmol) was added. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 24 hours. Reaction progress was checked via TLC plates developed in 40:60 acetone/hexane, as described hereinabove. The resulting product was crude thiopyridyl ester 9-TBDMS fluprostenol, compound xiv.

Anhydrous *o*-xylene (180 ml) was brought to reflux in a 1000 ml round-bottom flask under a nitrogen atmosphere. The crude compound xiv solution was added slowly to the refluxing xylene. The mixture was then refluxed for 3 hours under a nitrogen atmosphere. The reaction mixture was allowed to cool to room temperature and was stirred for 24 hours. The reaction progress was check with TLC plates developed in 40:60:1 acetone/hexane/acetic acid. The resulting crude lactone mixture was evaporated to give a viscous oil which was purified by chromatography on silica gel (300 g) packed and eluted with 1:4 acetone:dichloromethane.

Fractions containing the desired 9-TBDMS fluprostenol 1,15-lactone, compound xv, were combined and evaporated to give 370 mg of the desired compound as a colorless, viscous oil. The oil was transferred to a 50 ml round-bottom flask. A 5:95 mixture of 40% hydrofluoric acid (HF) in acetonitrile (10 ml) was added to the oil and

converted over a 2 hour period to a more polar product which co-migrated with the fluprostenol standard in 40:60 acetone:dichloromethane containing 0.5% acetic acid.

EXAMPLE 2

Synthesis of Latanoprost 1,15-lactone

Fig. 2 is an illustrative synthetic scheme used to prepare the 1,15-lactone of 17-phenyl-20,19,18-trinor $PGF_{2\alpha}$, or latanoprost 1,15-lactone (Structure VI).

The starting compound is a commercially available benzoate lactone diol, compound xx (See Cayman Chemical Catalog No. 70039). Compound xx, or 13,14-dihydro-15(R)-hydroxy-17-phenyl PG lactone 11-benzoate (6.0 g; 14.69 mmol) was dissolved in 60 ml of DMF (anhydrous) in a dry 500 ml round-bottom flask. Imidazole (3.03 g; 44.07 mmol) and TBDMS chloride (6.64 g, 44.07 mmol) was added slowly with stirring under a nitrogen atmosphere. The reaction mixture was stirred at room temperature overnight. The reaction mixture was then diluted with 200 ml of water and extracted with 300 ml of ethyl acetate acidified with 5% potassium hydrogen sulfate, and washed with 200 ml of brine. The combined aqueous mixture was then extracted 2x with 200 ml of ethyl acetate. The organic extract was washed twice with 200 ml of brine, dried over anhydrous sodium sulfate, filtered, and evaporated. The mixture was purified on 500 g of flash chromatography silica gel packed and eluted with 15:85 ethyl acetate/hexane. The product, mono-protected 15-TBDMS ether compound xxi, was a clear, colorless viscous oil. Mass of collected product was 7.42 g (96.6% yield).

Compound xxi showed a single spot at Rf=0.20 on silica gel-G TLC plates developed in 15:85 ethyl acetate/hexane and visualized with sulfuric acid/charring. An nmr scan (300 MHz-Bruker) run on compound xxi dissolved in deutero-chloroform revealed a doublet at 8.05 ppm (2H); multiplet at 7.6 ppm (1H); triplet at 7.5 ppm (2H); multiplet at 7.2-7.4 ppm (5H); a pair of multiplets at 5.1-5.2 ppm (2H); a multiplet at 3.7ppm (1H); broad multiplets from 2.3-3.0 ppm (8H); broad multiplets from 1.3-1.8 ppm (7H); single sharp singlet at 0.9 ppm (9H) and another sharp singlet at 0.02 ppm (6H), the latter two being the dimethyl silyl and the *t*-butyl methyl silyl groups, respectively.

Compound xxiii was dissolved in deutero-chloroform and an nmr (300 MHz-Bruker) was run, revealing a multiplet at 7.2-7.25 ppm (5H); a broad multiplet at 5.4 ppm (2H); three poorly defined multiplets at 3.7, 3.9 and 4.1ppm (3H) superimposed on a poorly defined broad absorbance spanning this entire area (1H); broad multiplets from 1.3-2.8 ppm (31H); single sharp singlet at 0.9 ppm (9H) and another sharp singlet at 0.02 ppm (6H), the latter two being the dimethyl silyl and the *t*-butyl methyl silyl groups, respectively.

A solution of compound xxiii (6.0 g;11.9 mmol) in 50 ml of anhydrous acetonitrile was placed in a 500 ml round-bottom flask and stirred at room temperature under a nitrogen atmosphere. The solution was cooled to 0°C and diisopropylethyl amine (6.2 ml; 35.7 mmol) was added, followed by 2.2 ml of iodomethane (35.7 mmol). The reaction mixture was stirred one hour at 0°C, and then 12 hours at room temperature. The mixture was then diluted with ethyl acetate, washed with water (200 ml) and then brine (200 ml x 3) and dried over anhydrous solid sodium sulfate. The solvent was evaporated under reduced pressure, and the crude product chromatographed over a 15 x 5 cm silica gel column packed and eluted with 40:60 ethyl acetate/hexane. Pure fractions were combined to give 5.5 g of the latanoprost methyl ester, 15-TBDMS ether compound xxiv.

Compound xxiv has an Rf of 0.35 on silica gel GF TLC plates eluted in 40:60 ethyl acetate:hexane and visualized with sulfuric acid/charring. Compound xxiv was dissolved in deutero-chloroform and an nmr (300 MHz-Bruker) was run, revealing a multiplet at 7.2-7.25 ppm (5H); a broad multiplet at 5.4 ppm (2H; three poorly defined multiplets at 3.7, 3.9 and 4.1 ppm (3H) a sharp singlet at 3.65 ppm (3H); broad multiplets from 1.3-2.8ppm (31H); single sharp singlet at 0.9 ppm (9H) and another sharp singlet at 0.02 ppm (6H), the latter two being the dimethyl silyl and the *t*-butyl methyl silyl groups, respectively.

A solution of compound xxiv (4.5 g; 8.7 mmol) in 100 ml of anhydrous dichloromethane was stirred at room temperature in a 250 ml round-bottom flask under a nitrogen atmosphere. Ethyl vinyl ether (8.3 ml; 10 equiv.) was added to the flask along with a catalytic amount (142 mg) of trichloroacetic acid. The reaction mixture was

dissolved in deutero-chloroform and an nmr (300 MHz-Bruker) was run, revealing a multiplet at 7.2-7.25 ppm (5H); a broad multiplet at 5.4 ppm (2H); a multiplet at 4.7 ppm (2H) representing the acetal methyne proton; three poorly defined multiplets at 3.7, 3.9 and 4.1 ppm (3H) superimposed on a poorly defined broad absorbance spanning this entire area (1H); a sharp singlet at 3.65 ppm (3H) and a multiplet at 3.5-3.6 ppm (4H); broad multiplets from 1.3-2.8 ppm (31H); peaks representative of the TBDMS group were notably absent.

A solution of compound xxvi (350 mg) in 5 ml of methanol and 1.5 ml THF was stirred at room temperature in a 100 ml pear-shaped flask. A 1 M solution of potassium hydroxide in water (1.5 ml) was added, and the mixture stirred at room temperature overnight. The reaction was then quenched with 10 ml of 5% potassium hydrogen sulfate. The mixture was extracted with ethyl acetate and the organic extract was rinsed with 50 ml brine followed by drying over solid anhydrous sodium sulfate. The volatile solvents were evaporated under reduced pressure, and the crude product was purified on a 72 x 2 cm silica gel column packed with acid-washed (pH=5.0) silica gel packed and eluted with 30:70 ethyl acetate/hexane. The pure fractions were combined to give 320 mg of the pure di-protected acid, 9,11-diethoxyethyl ether latanoprost free acid, compound xxvii.

Compound xxvii has an Rf of 0.27 on silica gel GF TLC plates eluted in 30:70:1 ethyl acetate/hexane/acetic acid and visualized with sulfuric acid/charring. Compound xxvii was dissolved in deutero-chloroform and an nmr (300 MHz-Bruker) was run, revealing a multiplet at 7.2-7.25 ppm (5H); a broad multiplet at 5.4 ppm (2H); a multiplet at 4.7 ppm (2H) representing the acetal methyne proton; three poorly defined multiplets at 3.7, 3.9 and 4.1 ppm (3H) superimposed on a poorly defined broad absorbance spanning this entire area (1H); a multiplet at 3.5–3.6 ppm (4H); broad multiplets from 1.3-2.8 ppm (31H).

A solution of compound xxvii (200 mg) in 5 ml of anhydrous xylene was stirred at room temperature in a 250 ml round-bottom flask under a nitrogen atmosphere. Triphenylphosphine (147 mg) and 108 mg of 2,2'-dipiridyl disulfide were added to the solution and the resulting mixture was stirred at room temperature for 18 hours. The

dissolved in deutero-chloroform and an nmr (300 MHz-Bruker) was run, revealing a multiplet at 7.2-7.3 ppm (5H); a broad multiplet at 5.1-5.45 ppm (3H); a multiplet from 3.6-4.2 ppm (3H); and broad multiplets from 0.9-2.8 ppm (29H). A mass spectrum run on the Finnegan LCQ mass spectrometer showed a molecular ion at m/e 373.0, and loss of H_2O (355.1) and 2 x H_2O (337.2).

EXPERIMENTAL RESULTS

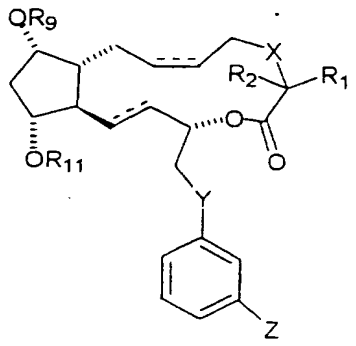
The ocular antihypertensive activity of $PGF_{2\alpha}$ analogs, including fluprostenol, is known to science. However, the ability of corneal esterases to act on the novel 1,15-lactones of 16- and 17-aryl-substituted analogs of $PGF_{2\alpha}$, thereby releasing the active free acid has not been shown. We therefore tested and documented the ability of corneal esterases to utilize fluprostenol 1,15-lactone (Structure IV; Example 1, compound xvi) as a substrate.

Enzymatic hydrolysis of fluprostenol 1,15-lactone by corneal esterase enzymes was documented by suspending 500 µg of fluprostenol 1,15-lactone in 10 ml of pH 7.4 phosphate buffered saline containing approximately 40 mg of freshly collected bovine corneal tissue. The mixture was incubated at 37°C and analyzed at 2 hour, 4 hour and 18 hour time intervals by Thin Layer Chromatography (TLC; Analtech silica gel G-60 plates) using 40:60 acetone:dichloromethane containing 0.5% acetic acid. The plates were visualized by spraying with vanillin dissolved in methanol and phosphoric acid, followed by charring on a hot plate. $PGF_{2\alpha}$ methyl ester, which is known to be hydrolyzed by corneal esterases, was subjected to the same procedure as a control.

The results are shown in Figs. 3A-3D which are images of chromatography plates developed at 2 hours (Figs. 3A and 3C) and 4 hours (Figs. 3B and 3D), respectively. Referring to Figs. 3A and 3B, lane 1 is the $PGF_{2\alpha}$ methyl ester standard, lane 2 is the mixture of $PGF_{2\alpha}$ methyl ester and bovine corneal tissue, and lane 3 is a $PGF_{2\alpha}$ free acid standard. Referring to Figs. 3C and 3D, lane 1 is the fluprostenol 1,15-lactone standard, lane 2 is the mixture of fluprostenol 1,15-lactone and bovine corneal tissue, and lane 3 is a fluprostenol free acid standard. By 4 hours, the release of the free acid by hydrolysis of the novel fluprostenol 1,15-lactone is clearly shown (See, Fig. 3D, comparing lanes 2 and 3).

WHAT IS CLAIMED IS:

1. A compound of the general formula:



Formula I

wherein X is O, S, NH or CH_2;

R_1 and R_2 are the same and are either H, CH_3 or F;

R_9 is H, or C_1-C_{20} straight chain, saturated or unsaturated or branched acyl;

R_{11} is H, or C_1-C_{20} straight chain, saturated or unsaturated or branched acyl;

represents any combination of a single bond, or a cis or trans double bond;

Z is H, Cl, Br, I, CF_3, CH_3, or C_1-C_{10} straight chain or branched alkyl;

Y is O, S, NH or CH_2.

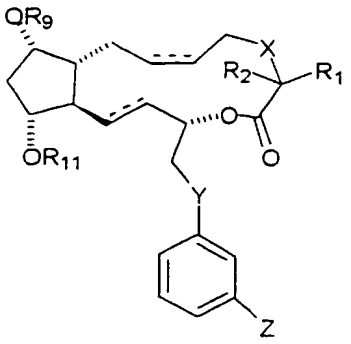
2. The compound of claim 1 wherein R_9 and R_{11} are H; Y is O, S, or NH; and Z is CH_3.

3. The compound of claim 1 wherein X is CH_2; R_1, R_2 is H; Y is O; and Z is CF_3.

4. The compound of claim 1 wherein X is CH_2; R_1, R_2 is H; Y is O; and Z is Cl.

5. The compound of claim 1 wherein X is CH_2; R_1, R_2 is H; Y is CH_2; and Z is H.

6. A method of treating increased intraocular pressure in the eye of a human or animal comprising the step of:



Formula I

wherein X is O, S, NH or CH_2;

R_1 and R_2 are the same and are either H, CH_3 or F;

R_9 is H, or C_1-C_{20} straight chain, saturated or unsaturated or branched acyl;

R_{11} is H, or C_1-C_{20} straight chain, saturated or unsaturated or branched acyl;

represents any combination of a single bond, or a cis or trans double bond;

Z is H, Cl, Br, I, CF_3, CH_3, or C_1-C_{10} straight chain or branched alkyl;

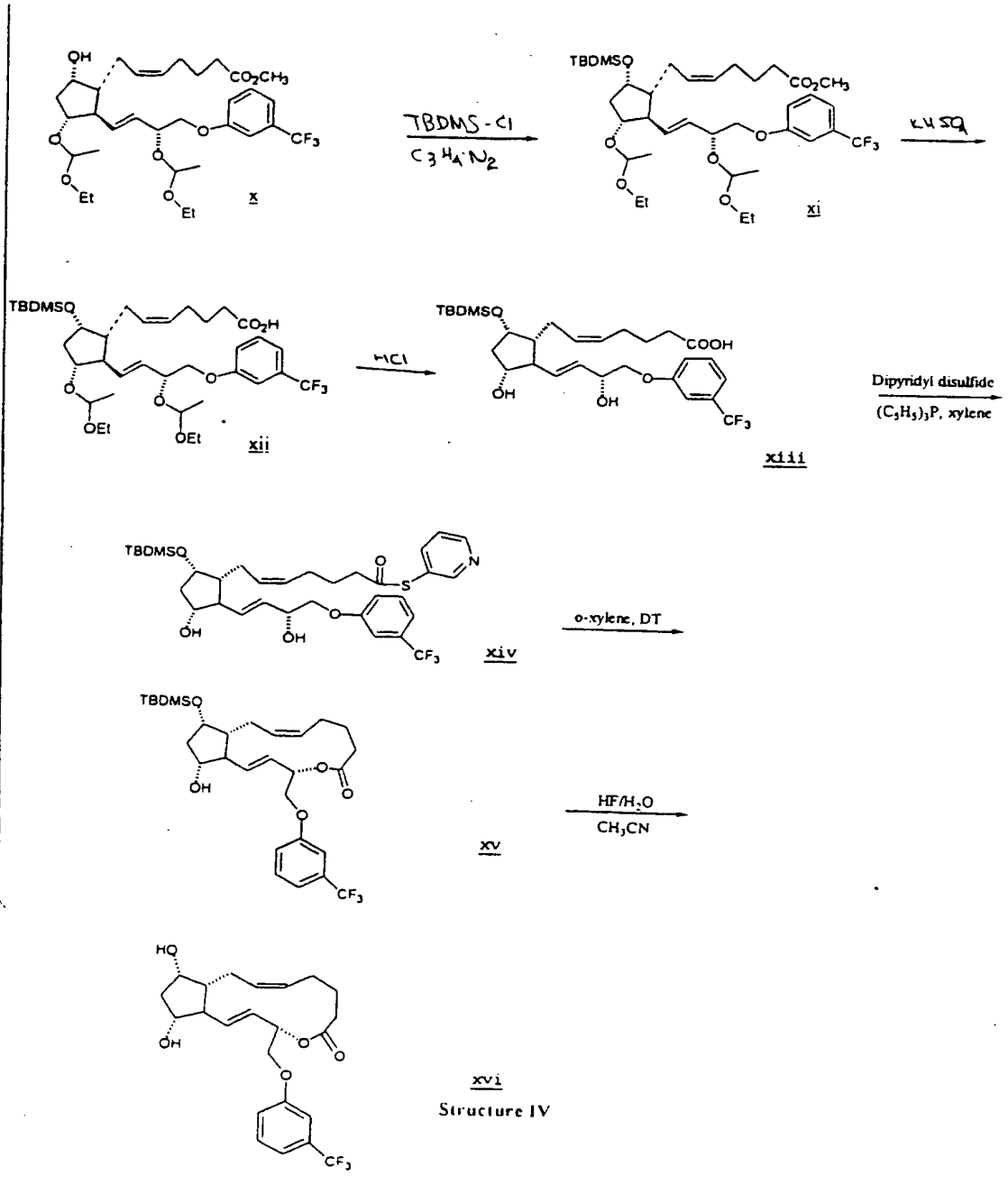
Y is O, S, NH or CH_2.

9. The topical ophthalmic composition of claim 8 wherein the compound is selected from the group consisting of a 1,15-lactone of fluprostenol, a 1,15-lactone of cloprostenol, and a 1,15-lactone of latanoprost.

10. A topical formulation for treating increased intraocular pressure comprising the following ingredients by weight percent:

Fluprostenol 1,15-lactone	0.002
Dextran 70	0.1
Hydroxypropyl Methylcellulose	0.3
Sodium Chloride	0.77
Potassium Chloride	0.12
Disodium EDTA	0.05
Benzalkonium Chloride	0.01
HCl and/or NaOH	to pH=7.0-7.6
Purified water	q.s. to 100%

FIG. 1



Structure IV

Control ($PGF_{2\alpha}$ methyl ester) and bovine cornea

Lane 1: $PGF_{2\alpha}$ methyl ester (standard)
Lane 2: $PGF_{2\alpha}$ methyl ester and bovine cornea
Lane 3: $PGF_{2\alpha}$ free acid (standard)

FIG. 3A

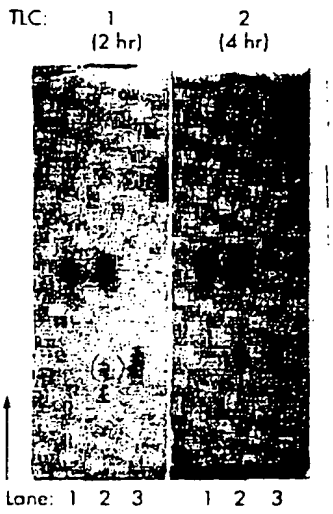
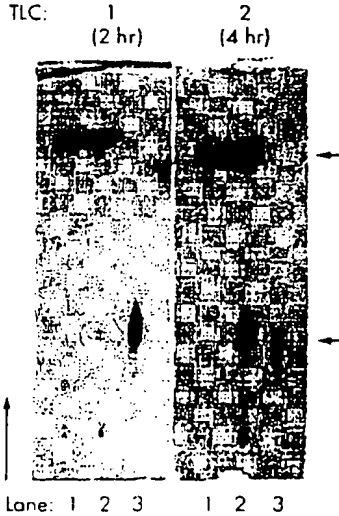


FIG. 3B

Fluprostenol 1,15-Lactone and bovine cornea

Lane 1: 1,15-Lactone (standard)
Lane 2: 1,15- Lactone and bovine cornea
Lane 3: Fluprostenol free acid (standard)

FIG. 3C



Fluprostenol 1,15-Lactone

FIG. 3D

Fluprostenol free acid

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/US 01/03559

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D313/00 A61P27/06 A61K31/365

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; UPJOHN CO., USA: "Prostaglandin lactones" retrieved from STN Database accession no. 88:104768 XP002166932 the whole document -& JP 52 001034 A (UPJOHN CO., USA) 6 January 1977 (1977-01-06) ---	1,3-5
X	BUNDY, GORDON L. ET AL: "Synthesis and biological activity of prostaglandin lactones" J. MED. CHEM. (1983), 26(8), 1089-99 , XP000999619 abstract ---	1,5
Y	examples 41,42; table III ---	1-10
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 May 2001	30/05/2001

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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Filed on 1 February 2000 (01.02.2000)

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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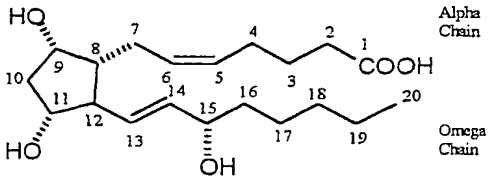
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: INTERNAL 1,15-LACTONES OF FLUPROSTENOL AND RELATED PROSTAGLANDIN $F_{2\alpha}$ ANALOGS AND THEIR USE IN THE TREATMENT OF GLAUCOMA AND INTRAOCULAR HYPERTENSION

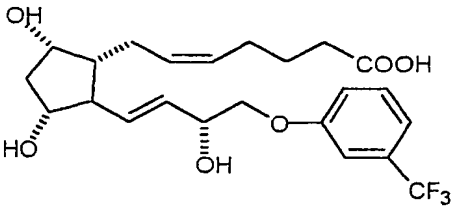
(57) Abstract: Novel derivatives of prostaglandin compounds of the F-series (PGF), specifically macrocyclic internal 1,15-lactones of fluprostenol and related PGF analogs, such as cloprostenol or latanoprost. The novel analogs can be formulated into ophthalmic solutions and topically applied for the treatment of the increased intraocular pressure caused by glaucoma and the reduction of ocular hypertension.

WO 01/057015 A1

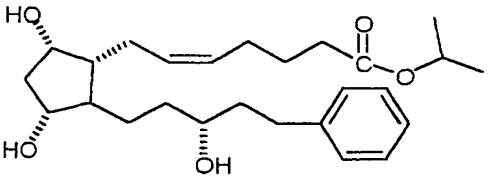
A number of simple $PGF_{2\alpha}$ analog esters have been found to be potent and selective agents useful for the treatment of ocular hypertension. For example, latanoprost is the isopropyl ester of 13,14-dihydro-17-phenyl-18,19,20-trinor $PGF_{2\alpha}$ and is widely marketed for the clinical treatment of glaucoma under the trade name Xalatan. See monograph 5387, page 918 of *The Merck Index*, 12th edition (1996). Likewise, the isopropyl ester of fluprostenol and of similar $PGF_{2\alpha}$ analogs, such as cloprostenol, are specifically claimed as ocular antihypertensive agents in U.S. patent 5,665,773. The structures of naturally-occurring $PGF_{2\alpha}$ (Structure I), fluprostenol (Structure II), and latanoprost (Structure III) are shown hereinbelow. For a review of these agents, see Lindén and Alm, *Drugs and Aging*, Vol.14, No. 5, pp. 387-398 (1999).



Structure I



Structure II



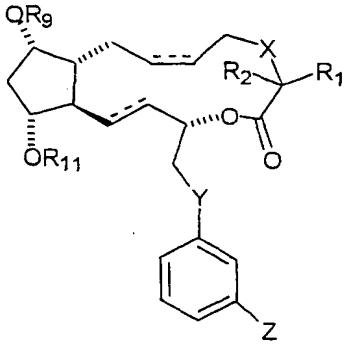
Structure III

prostaglandins, and in particular internal 1,15-lactones of $PGF_{2\alpha}$ analogs, such as the 16-aryloxy prostaglandin analogs, illustratively fluprostenol or cloprostenol.

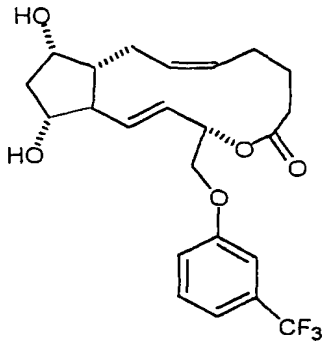
We have discovered that it is possible to form an internal ester of $PGF_{2\alpha}$ analogs by creating a carbon-oxygen bond between the alcohol group at C-15 and the C-1 carboxylic acid. This creates a macrocyclic lactone that has novel and desirable characteristics. In fact, some of the novel analogs form highly crystalline structures that are easy to formulate into ophthalmic solutions, for example. The hydrolysis of these $PGF_{2\alpha}$ analog 1,15-lactones releases only the active $PGF_{2\alpha}$ analog free acid, without the production of a small aliphatic alcohol coproduct. Thus, these compounds are ideal and unique prodrugs for the treatment of glaucoma and other disorders causing an increase in intraocular pressure in the eyes of humans or animals.

For the purposes of this patent, the term "prostaglandin" is intended to mean any one of the prostanoic acid derivatives which include the ring type A, B, C, D, E, F, G, H, I, J and K, but most particularly those of the F-type. The term "derivative" is intended to mean all compounds which have a chemical affinity, resemblance, or structural character which clearly associates them with the prostanoids and in particular, prostanoic acid or $PGF_{2\alpha}$. The term "analog" is intended to mean any somewhat modified version of a natural product, in this case a prostaglandin, or a related synthetic analog, wherein a number of atoms such as carbon, hydrogen, oxygen or heteroatoms such as nitrogen, sulfur or halide have been added or deleted from the parent structure, so as to yield a new molecular compound.

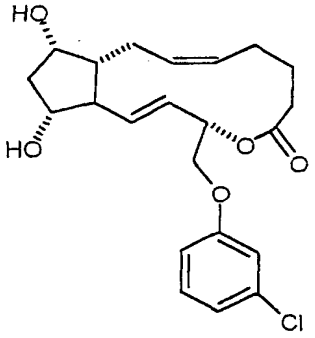
The compounds of the present invention have the general Formula I:



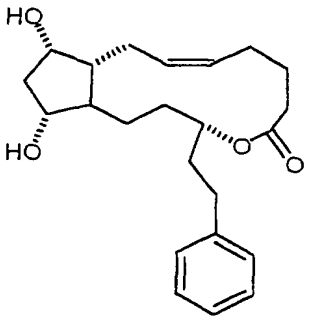
Formula I



Structure IV



Structure V



Structure VI

osmolarity adjusting agents, such as sodium chloride (NaCl) or potassium chloride (KCl), as is known in the art. Other ingredients which may be desirable to use in the ophthalmic preparations of the present invention include preservatives, co-solvents and viscosity building agents.

Ophthalmic products are typically packaged in multidose form, which generally require the addition of preservatives to prevent microbial contamination during use. Suitable preservatives include: benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, ONAMER M®, or other agents known to those skilled in the art. Such preservatives are typically employed at a concentration between about 0.001% and 1.0% by weight.

Prostaglandins, and particularly ester derivatives, typically have limited solubility in water and therefore may require a surfactant or other appropriate co-solvent in the composition. Such co-solvents include: Polysorbate 20, 60 and 80; Pluronic F-68, F-84 and P-103; Tyloxapol®; Cremophor® EL; sodium dodecyl sulfate; glycerol; PEG 400; propylene glycol; cyclodextrins; or other agents known to those skilled in the art. Such co-solvents are typically employed at a concentration between about 0.01% and about 2% by weight.

Viscosity greater than that of simple aqueous solutions may be desirable to increase ocular absorption of the active compound, to decrease variability in dispensing the formulations, to decrease physical separation of components of a suspension or emulsion of formulation and/or otherwise to improve the ophthalmic formulation. Such viscosity building agents include, for example, polyvinyl alcohol, polyvinyl pyrrolidone, methyl cellulose, hydroxy propyl methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxy propyl cellulose or other agents known to those skilled in the art. Such agents are typically employed at a concentration between about 0.01% and about 2% by weight.

In a specific illustrative embodiment, a topical formulation for treating increased intraocular pressure, in accordance with the invention, comprises:

A 4 L, 3-neck round-bottom flask was dried in an oven at 110°C overnight and fitted with an addition funnel, an overhead stirrer and a dry nitrogen gas inlet tube. Sodium hydride (NaH; 0.617 moles; 14.8 g) as a dry powder was suspended in 1.5 L of anhydrous tetrahydrofuran (THF). The mixture was cooled to 0°C with an external ice bath and 75 ml (100 g; 0.617 moles) of *m*-trifluoromethyl cresol was added dropwise and stirred one hour at 0°C and 2 hours at 22°C. The reaction mixture was then cooled to 0°C and (47.3 ml; 0.5 moles) of methyl bromoacetate was added dropwise. The mixture was stirred an additional 2 hours at 0°C and 1 hour at 22°C. 1.5 L of ethyl acetate was then added and the mixture transferred to a 6 L separatory funnel. 2 L of water was added and the layers were separated. The organic layer was washed twice with 1 L of brine, dried over solid anhydrous sodium sulfate, and the solvents evaporated to give 150 g of the trifluoromethyl compound ii as a yellow oil.

This product may be used directly in the next step of the synthesis, or more reliably may be purified by flash chromatography and used in a more purified form. The trifluoromethyl compound ii (86 g) is dissolved in 1.5 L of anhydrous THF and placed in a 2 L addition funnel over a 3-neck, 3 L round-bottom flask under dry nitrogen. Dimethyl methylphosphonate (63.3 ml) was added directly to the 3 L flask along with 1.2 L of anhydrous THF and cooled to -78°C with an external dry ice / acetone bath while stirring well with a mechanical stirrer. 2.5 M n-butyl lithium (217.6 ml) was added dropwise. The mixture was stirred at -78° for 90 minutes, and the solution of compound ii was then added dropwise over 30 minutes. The reaction was maintained an additional 4 hours at -78°C then stirred at ambient temperature overnight. The reaction mixture was then acidified with 2 L of 5% potassium hydrogen sulfate ($KHSO_4$) and transferred to a 6 L separatory funnel. It was diluted with 1.5 L of ethyl acetate, and the aqueous layer extracted once with 1 L of ethyl acetate and discarded. The organic layers were combined and washed with 1 L portions of brine until neutral, then dried over solid sodium sulfate and the solvent evaporated to give 237 g of yellow oil. This oil was purified by flash chromatography on silicic acid packed and eluted with 20:80 hexane:ethyl acetate. Pure fractions were combined and evaporated to give 175 g of the phosphonate compound iii.

sulfate. Removal of solvent on a rotary evaporator under reduced pressure afforded 200 g of an oil. The oil was purified on a silica gel column using 10:90 acetone:hexane as the eluant to afford 60 g of the benzoate alcohol compound v.

A solution of the benzoate alcohol compound v (60 g; 126 mmol) in 900 ml of methanol was placed in a 2 L round-bottom flask. Potassium carbonate (K_2CO_3; 21g; 159 mmol) was added and the reaction mixture was stirred at ambient temperature for 90 minutes. The reaction mixture was cooled to 0°C and acidified to pH 6 with 5% potassium hydrogen sulfate. The reaction mixture was diluted with 1500 ml of brine and extracted twice with 1 L of ethyl acetate. The organic layers were combined and washed with brine until it had a neutral pH. The organic phase was dried over sodium sulfate and concentrated on a rotary evaporator under reduced pressure to afford an oil which was purified on a silica gel column using 90:10 ethyl acetate:hexane as the eluant to furnish the desired lactone diol compound vi.

A 3 L jacketed-flask was equipped with a mechanical stirrer and a temperature microprocessor. The flask was charged with the lactone diol compound vi (~148 g; 0.397 moles) and approximately 2000 ml of methylene chloride under an atmosphere of nitrogen. This mixture was stirred until dissolved.

Approximately 7 equivalents of ethyl vinyl ether (266 ml; 2.779 moles) was added to the flask followed by the addition of approximately 0.1 equivalents of trichloroacetic acid (6.49 g; 0.0397 moles). The reaction mixture was stirred at room temperature until the reaction was judged to be complete by monitoring the reaction progress with thin layer chromatography (TLC). In this case, the reaction mixture was spotted on a silica gel TLC plate alongside a spot of the starting material. The spotted plate was placed into a TLC tank containing 80% ethyl acetate, 20% hexane (v/v). To develop, the TLC plate was sprayed with a 50:50 mixture of sulfuric acid and water (v/v) and heated. In some instances, it may be necessary to heat the reaction mixture to 30°C ± 5° for the reaction to go to completion.

While the reaction mixture is going to completion, a 10% potassium bicarbonate solution was prepared by combining approximately 10 g of potassium bicarbonate with approximately 100 ml of tap water in a 250 ml Erlenmeyer flask and swirling until

was spotted on a silica gel TLC plate alongside a spot of the starting material. The spotted plate was placed into a TLC tank containing 50% ethyl acetate, 50% hexane (v/v). To develop, the TLC plate was sprayed with a 50:50 mixture of sulfuric acid and water (v/v) and charred with heat. Once the reaction was judged to be complete, the heating circulator was turned off.

Approximately 0.31 equivalents of tap water (to DIBAL) and approximately 700 ml THF was combined in a 1 L Erlenmeyer flask and chilled. Excess DIBAL was decomposed by adding the chilled mixture of water and THF to the stirring solution through the addition funnel attached to the 6 L reaction vessel. The water/THF solution should be added dropwise and slowly. In particular, the first 50-100 ml should be added very slowly because foaming can occur. The temperature was allowed to rise during the addition. Once the addition is complete, the temperature should be between 0°C to -45°C.

Using the Fisher circulator, the reaction was warmed to approximately 20°C and then stirred for about 1 hour. A temperature of about 28°C ±10°C should be maintained. After about 30 minutes, the reaction tends to heat up because the salts are hydrating. Over the 1 hour period, the reaction mixture went from a dull yellowish-brown color to a titanium white slurry.

Approximately 990 ml toluene and approximately 660 ml THF was combined in a separate flask. Approximately 2 inches of celite 545 was placed in a 2 L fritted-funnel and enough of the mixture was poured over the top of the celite so that it was totally covered. Once the reaction was complete, the slurry was filtered over the celite using a water aspirator for suction. A stream of nitrogen was aimed at the filter funnel during filtration. The filter cake and reaction vessel was washed with the toluene/THF. The filter cake was discarded. The solvent was evaporated to give lactol compound viii as a viscous yellow oil which was used directly, without purification in the next step.

4-Carboxybutyl triphenylphosphine bromide (8.57 g; 19.34) was suspended in 30 ml of THF (anhydrous). Potassium *tert*-butoxide (38.68 ml; 38.68 mmol) was slowly added to this suspension. The reaction mixture was stirred at room temperature for 45 minutes and then cooled down to -10°C with ice/NaCl. Subsequently, lactol compound

sulfate and evaporated. The residue was purified on an acid-washed silica gel column using 10:90 ethyl acetate/hexane as eluent. Mass of collected product, 9-TBDMS-fluprostenol 11,15-diethoxyethyl ether, compound xii, was 5.64 (95.40% yield).

Compound xii (3 g) was dissolved in 100 ml of THF in a 500 ml round-bottom flask and stirred, under a nitrogen atmosphere, at room temperature. Following the addition of 0.5 M hydrochloric acid (2.0 ml), the reaction mixture was stirred at ambient temperature for 2 hours. The reaction mixture was then diluted with ethyl acetate, saturated with brine, and extracted once with ethyl acetate. The combined organic solvents were dried over anhydrous sodium sulfate and the solvents were removed under reduced pressure to give 2.57 grams of fluprostenol 9-TBDMS ether, compound xiii, as a viscous oil.

Compound xiii (2.57 g; 5.9 mmol) was dissolved in 30 ml of anhydrous (oxygen free) xylene. To this solution, 2,2'-dipyridyl bisulfide (1.59 g; 7.2 mmol) and triphenylphosphine (1.89 g; 7.2 mmol) was added. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 24 hours. Reaction progress was checked via TLC plates developed in 40:60 acetone/hexane, as described hereinabove. The resulting product was crude thiopyridyl ester 9-TBDMS fluprostenol, compound xiv.

Anhydrous *o*-xylene (180 ml) was brought to reflux in a 1000 ml round-bottom flask under a nitrogen atmosphere. The crude compound xiv solution was added slowly to the refluxing xylene. The mixture was then refluxed for 3 hours under a nitrogen atmosphere. The reaction mixture was allowed to cool to room temperature and was stirred for 24 hours. The reaction progress was check with TLC plates developed in 40:60:1 acetone/hexane/acetic acid. The resulting crude lactone mixture was evaporated to give a viscous oil which was purified by chromatography on silica gel (300 g) packed and eluted with 1:4 acetone:dichloromethane.

Fractions containing the desired 9-TBDMS fluprostenol 1,15-lactone, compound xv, were combined and evaporated to give 370 mg of the desired compound as a colorless, viscous oil. The oil was transferred to a 50 ml round-bottom flask. A 5:95 mixture of 40% hydrofluoric acid (HF) in acetonitrile (10 ml) was added to the oil and

converted over a 2 hour period to a more polar product which co-migrated with the fluprostenol standard in 40:60 acetone:dichloromethane containing 0.5% acetic acid.

EXAMPLE 2

Synthesis of Latanoprost 1,15-lactone

Fig. 2 is an illustrative synthetic scheme used to prepare the 1,15-lactone of 17-phenyl-20,19,18-trinor $PGF_{2\alpha}$, or latanoprost 1,15-lactone (Structure VI).

The starting compound is a commercially available benzoate lactone diol, compound xx (See Cayman Chemical Catalog No. 70039). Compound xx, or 13,14-dihydro-15(R)-hydroxy-17-phenyl PG lactone 11-benzoate (6.0 g; 14.69 mmol) was dissolved in 60 ml of DMF (anhydrous) in a dry 500 ml round-bottom flask. Imidazole (3.03 g; 44.07 mmol) and TBDMS chloride (6.64 g, 44.07 mmol) was added slowly with stirring under a nitrogen atmosphere. The reaction mixture was stirred at room temperature overnight. The reaction mixture was then diluted with 200 ml of water and extracted with 300 ml of ethyl acetate acidified with 5% potassium hydrogen sulfate, and washed with 200 ml of brine. The combined aqueous mixture was then extracted 2x with 200 ml of ethyl acetate. The organic extract was washed twice with 200 ml of brine, dried over anhydrous sodium sulfate, filtered, and evaporated. The mixture was purified on 500 g of flash chromatography silica gel packed and eluted with 15:85 ethyl acetate/hexane. The product, mono-protected 15-TBDMS ether compound xxi, was a clear, colorless viscous oil. Mass of collected product was 7.42 g (96.6% yield).

Compound xxi showed a single spot at Rf=0.20 on silica gel-G TLC plates developed in 15:85 ethyl acetate/hexane and visualized with sulfuric acid/charring. An nmr scan (300 MHz-Bruker) run on compound xxi dissolved in deutero-chloroform revealed a doublet at 8.05 ppm (2H); multiplet at 7.6 ppm (1H); triplet at 7.5 ppm (2H); multiplet at 7.2-7.4 ppm (5H); a pair of multiplets at 5.1-5.2 ppm (2H); a multiplet at 3.7ppm (1H); broad multiplets from 2.3-3.0 ppm (8H); broad multiplets from 1.3-1.8 ppm (7H); single sharp singlet at 0.9 ppm (9H) and another sharp singlet at 0.02 ppm (6H), the latter two being the dimethyl silyl and the *t*-butyl methyl silyl groups, respectively.

Compound xxiii was dissolved in deutero-chloroform and an nmr (300 MHz-Bruker) was run, revealing a multiplet at 7.2-7.25 ppm (5H); a broad multiplet at 5.4 ppm (2H); three poorly defined multiplets at 3.7, 3.9 and 4.1ppm (3H) superimposed on a poorly defined broad absorbance spanning this entire area (1H); broad multiplets from 1.3-2.8 ppm (31H); single sharp singlet at 0.9 ppm (9H) and another sharp singlet at 0.02 ppm (6H), the latter two being the dimethyl silyl and the *t*-butyl methyl silyl groups, respectively.

A solution of compound xxiii (6.0 g;11.9 mmol) in 50 ml of anhydrous acetonitrile was placed in a 500 ml round-bottom flask and stirred at room temperature under a nitrogen atmosphere. The solution was cooled to 0°C and diisopropylethyl amine (6.2 ml; 35.7 mmol) was added, followed by 2.2 ml of iodomethane (35.7 mmol). The reaction mixture was stirred one hour at 0°C, and then 12 hours at room temperature. The mixture was then diluted with ethyl acetate, washed with water (200 ml) and then brine (200 ml x 3) and dried over anhydrous solid sodium sulfate. The solvent was evaporated under reduced pressure, and the crude product chromatographed over a 15 x 5 cm silica gel column packed and eluted with 40:60 ethyl acetate/hexane. Pure fractions were combined to give 5.5 g of the latanoprost methyl ester, 15-TBDMS ether compound xxiv.

Compound xxiv has an Rf of 0.35 on silica gel GF TLC plates eluted in 40:60 ethyl acetate:hexane and visualized with sulfuric acid/charring. Compound xxiv was dissolved in deutero-chloroform and an nmr (300 MHz-Bruker) was run, revealing a multiplet at 7.2-7.25 ppm (5H); a broad multiplet at 5.4 ppm (2H; three poorly defined multiplets at 3.7, 3.9 and 4.1 ppm (3H) a sharp singlet at 3.65 ppm (3H); broad multiplets from 1.3-2.8ppm (31H); single sharp singlet at 0.9 ppm (9H) and another sharp singlet at 0.02 ppm (6H), the latter two being the dimethyl silyl and the *t*-butyl methyl silyl groups, respectively.

A solution of compound xxiv (4.5 g; 8.7 mmol) in 100 ml of anhydrous dichloromethane was stirred at room temperature in a 250 ml round-bottom flask under a nitrogen atmosphere. Ethyl vinyl ether (8.3 ml; 10 equiv.) was added to the flask along with a catalytic amount (142 mg) of trichloroacetic acid. The reaction mixture was

dissolved in deutero-chloroform and an nmr (300 MHz-Bruker) was run, revealing a multiplet at 7.2-7.25 ppm (5H); a broad multiplet at 5.4 ppm (2H); a multiplet at 4.7 ppm (2H) representing the acetal methyne proton; three poorly defined multiplets at 3.7, 3.9 and 4.1 ppm (3H) superimposed on a poorly defined broad absorbance spanning this entire area (1H); a sharp singlet at 3.65 ppm (3H) and a multiplet at 3.5-3.6 ppm (4H); broad multiplets from 1.3-2.8 ppm (31H); peaks representative of the TBDMS group were notably absent.

A solution of compound xxvi (350 mg) in 5 ml of methanol and 1.5 ml THF was stirred at room temperature in a 100 ml pear-shaped flask. A 1 M solution of potassium hydroxide in water (1.5 ml) was added, and the mixture stirred at room temperature overnight. The reaction was then quenched with 10 ml of 5% potassium hydrogen sulfate. The mixture was extracted with ethyl acetate and the organic extract was rinsed with 50 ml brine followed by drying over solid anhydrous sodium sulfate. The volatile solvents were evaporated under reduced pressure, and the crude product was purified on a 72 x 2 cm silica gel column packed with acid-washed (pH=5.0) silica gel packed and eluted with 30:70 ethyl acetate/hexane. The pure fractions were combined to give 320 mg of the pure di-protected acid, 9,11-diethoxyethyl ether latanoprost free acid, compound xxvii.

Compound xxvii has an Rf of 0.27 on silica gel GF TLC plates eluted in 30:70:1 ethyl acetate/hexane/acetic acid and visualized with sulfuric acid/charring. Compound xxvii was dissolved in deutero-chloroform and an nmr (300 MHz-Bruker) was run, revealing a multiplet at 7.2-7.25 ppm (5H); a broad multiplet at 5.4 ppm (2H); a multiplet at 4.7 ppm (2H) representing the acetal methyne proton; three poorly defined multiplets at 3.7, 3.9 and 4.1 ppm (3H) superimposed on a poorly defined broad absorbance spanning this entire area (1H); a multiplet at 3.5–3.6 ppm (4H); broad multiplets from 1.3-2.8 ppm (31H).

A solution of compound xxvii (200 mg) in 5 ml of anhydrous xylene was stirred at room temperature in a 250 ml round-bottom flask under a nitrogen atmosphere. Triphenylphosphine (147 mg) and 108 mg of 2,2'-dipiridyl disulfide were added to the solution and the resulting mixture was stirred at room temperature for 18 hours. The

dissolved in deutero-chloroform and an nmr (300 MHz-Bruker) was run, revealing a multiplet at 7.2-7.3 ppm (5H); a broad multiplet at 5.1-5.45 ppm (3H); a multiplet from 3.6-4.2 ppm (3H); and broad multiplets from 0.9-2.8 ppm (29H). A mass spectrum run on the Finnegan LCQ mass spectrometer showed a molecular ion at m/e 373.0, and loss of H_2O (355.1) and 2 x H_2O (337.2).

EXPERIMENTAL RESULTS

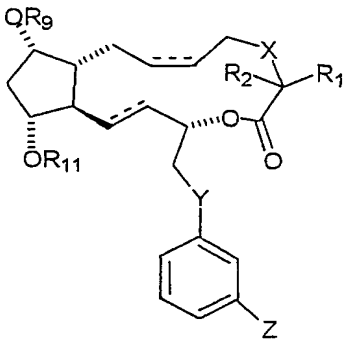
The ocular antihypertensive activity of $PGF_{2\alpha}$ analogs, including fluprostenol, is known to science. However, the ability of corneal esterases to act on the novel 1,15-lactones of 16- and 17-aryl-substituted analogs of $PGF_{2\alpha}$, thereby releasing the active free acid has not been shown. We therefore tested and documented the ability of corneal esterases to utilize fluprostenol 1,15-lactone (Structure IV; Example 1, compound xvi) as a substrate.

Enzymatic hydrolysis of fluprostenol 1,15-lactone by corneal esterase enzymes was documented by suspending 500 μg of fluprostenol 1,15-lactone in 10 ml of pH 7.4 phosphate buffered saline containing approximately 40 mg of freshly collected bovine corneal tissue. The mixture was incubated at 37°C and analyzed at 2 hour, 4 hour and 18 hour time intervals by Thin Layer Chromatography (TLC; Analtech silica gel G-60 plates) using 40:60 acetone:dichloromethane containing 0.5% acetic acid. The plates were visualized by spraying with vanillin dissolved in methanol and phosphoric acid, followed by charring on a hot plate. $PGF_{2\alpha}$ methyl ester, which is known to be hydrolyzed by corneal esterases, was subjected to the same procedure as a control.

The results are shown in Figs. 3A-3D which are images of chromatography plates developed at 2 hours (Figs. 3A and 3C) and 4 hours (Figs. 3B and 3D), respectively. Referring to Figs. 3A and 3B, lane 1 is the $PGF_{2\alpha}$ methyl ester standard, lane 2 is the mixture of $PGF_{2\alpha}$ methyl ester and bovine corneal tissue, and lane 3 is a $PGF_{2\alpha}$ free acid standard. Referring to Figs. 3C and 3D, lane 1 is the fluprostenol 1,15-lactone standard, lane 2 is the mixture of fluprostenol 1,15-lactone and bovine corneal tissue, and lane 3 is a fluprostenol free acid standard. By 4 hours, the release of the free acid by hydrolysis of the novel fluprostenol 1,15-lactone is clearly shown (See, Fig. 3D, comparing lanes 2 and 3).

WHAT IS CLAIMED IS:

1. A compound of the general formula:



Formula I

wherein X is O, S, NH or CH_2;

R_1 and R_2 are the same and are either H, CH_3 or F;

R_9 is H, or C_1-C_{20} straight chain, saturated or unsaturated or branched acyl;

R_{11} is H, or C_1-C_{20} straight chain, saturated or unsaturated or branched acyl;

represents any combination of a single bond, or a cis or trans double bond;

Z is H, Cl, Br, I, CF_3, CH_3, or C_1-C_{10} straight chain or branched alkyl;

Y is O, S, NH or CH_2.

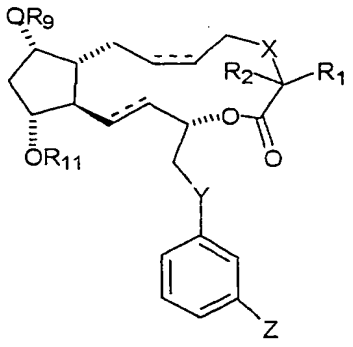
2. The compound of claim 1 wherein R_9 and R_{11} are H; Y is O, S, or NH; and Z is CH_3.

3. The compound of claim 1 wherein X is CH_2; R_1, R_2 is H; Y is O; and Z is CF_3.

4. The compound of claim 1 wherein X is CH_2; R_1, R_2 is H; Y is O; and Z is Cl.

5. The compound of claim 1 wherein X is CH_2; R_1, R_2 is H; Y is CH_2; and Z is H.

6. A method of treating increased intraocular pressure in the eye of a human or animal comprising the step of:



Formula I

wherein X is O, S, NH or CH_2;

R_1 and R_2 are the same and are either H, CH_3 or F;

R_9 is H, or C_1-C_{20} straight chain, saturated or unsaturated or branched acyl;

R_{11} is H, or C_1-C_{20} straight chain, saturated or unsaturated or branched acyl;

represents any combination of a single bond, or a cis or trans double bond;

Z is H, Cl, Br, I, CF_3, CH_3, or C_1-C_{10} straight chain or branched alkyl;

Y is O, S, NH or CH_2.

9. The topical ophthalmic composition of claim 8 wherein the compound is selected from the group consisting of a 1,15-lactone of fluprostenol, a 1,15-lactone of cloprostenol, and a 1,15-lactone of latanoprost.

10. A topical formulation for treating increased intraocular pressure comprising the following ingredients by weight percent:

Fluprostenol 1,15-lactone	0.002
Dextran 70	0.1
Hydroxypropyl Methylcellulose	0.3
Sodium Chloride	0.77
Potassium Chloride	0.12
Disodium EDTA	0.05
Benzalkonium Chloride	0.01
HCl and/or NaOH	to pH=7.0-7.6
Purified water	q.s. to 100%

FIG. 1B

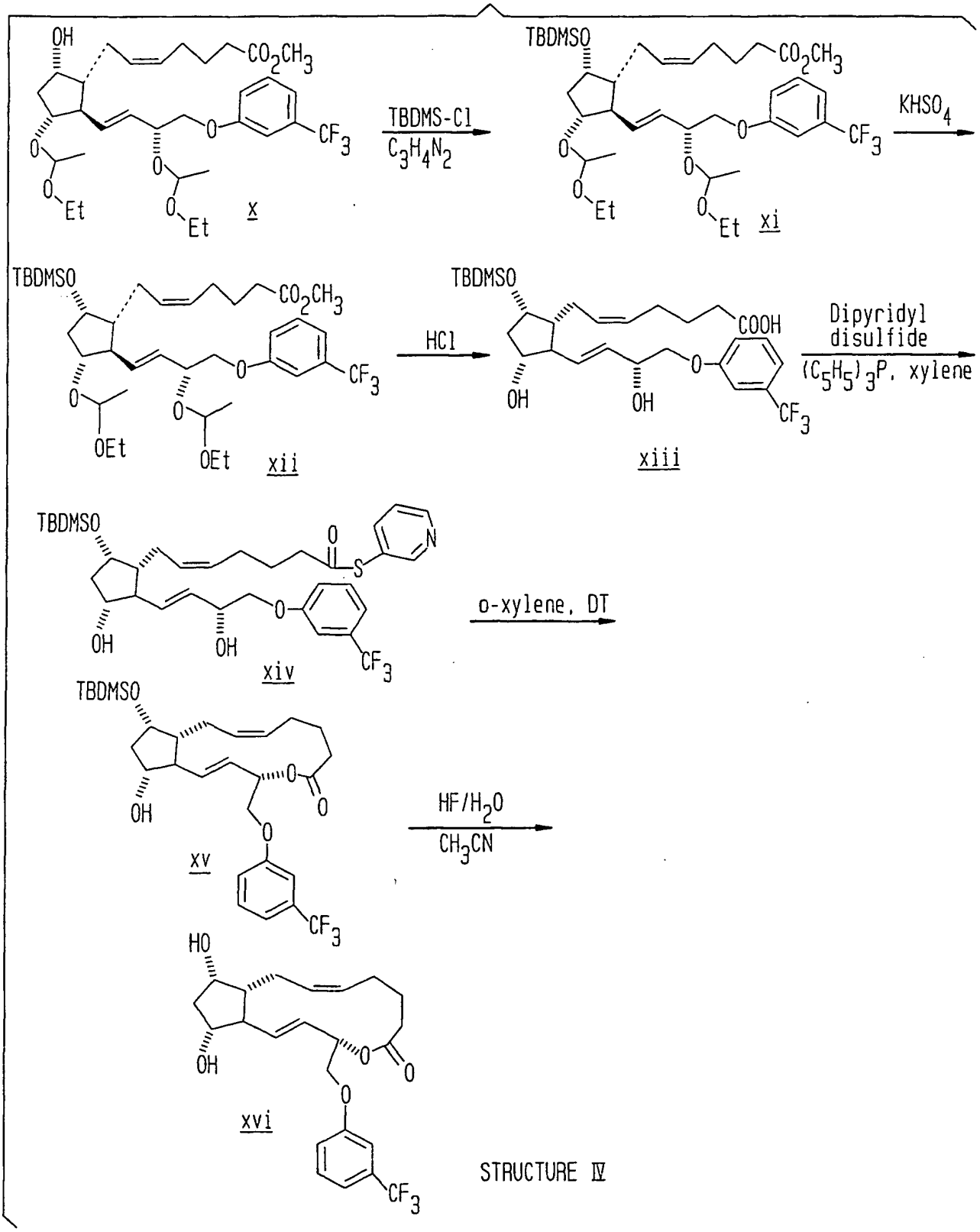


FIG. 3A

CONTROL ($PGF_{2\alpha}$ METHYL ESTER) AND BOVINE CORNEA

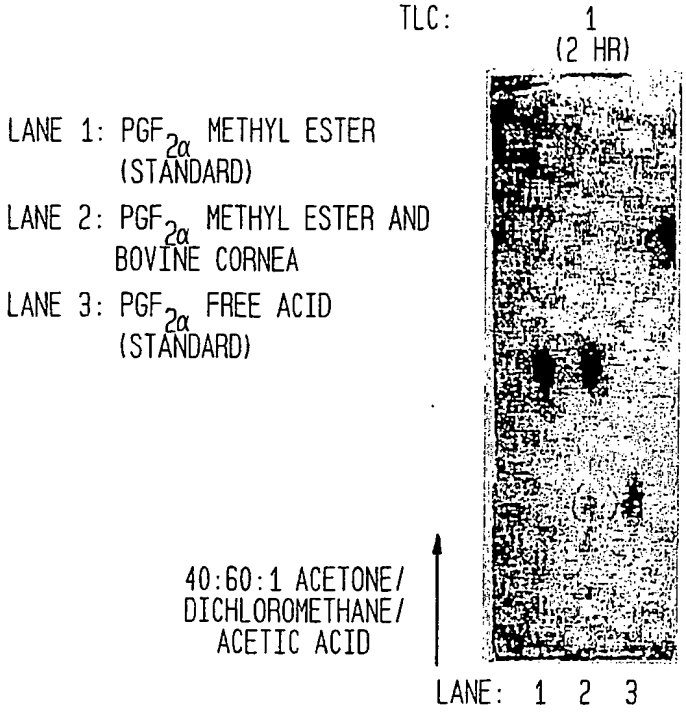


FIG. 3B

CONTROL ($PGF_{2\alpha}$ METHYL ESTER) AND BOVINE CORNEA

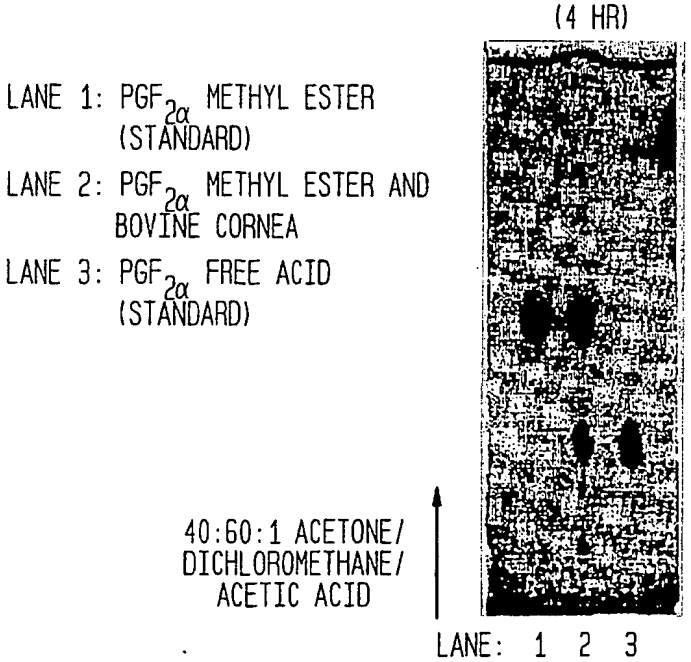


FIG. 4A

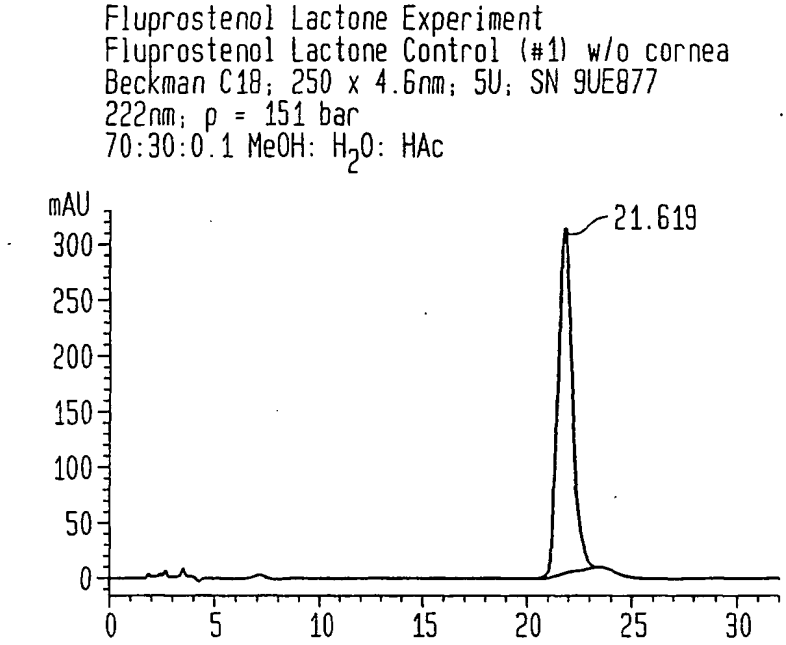
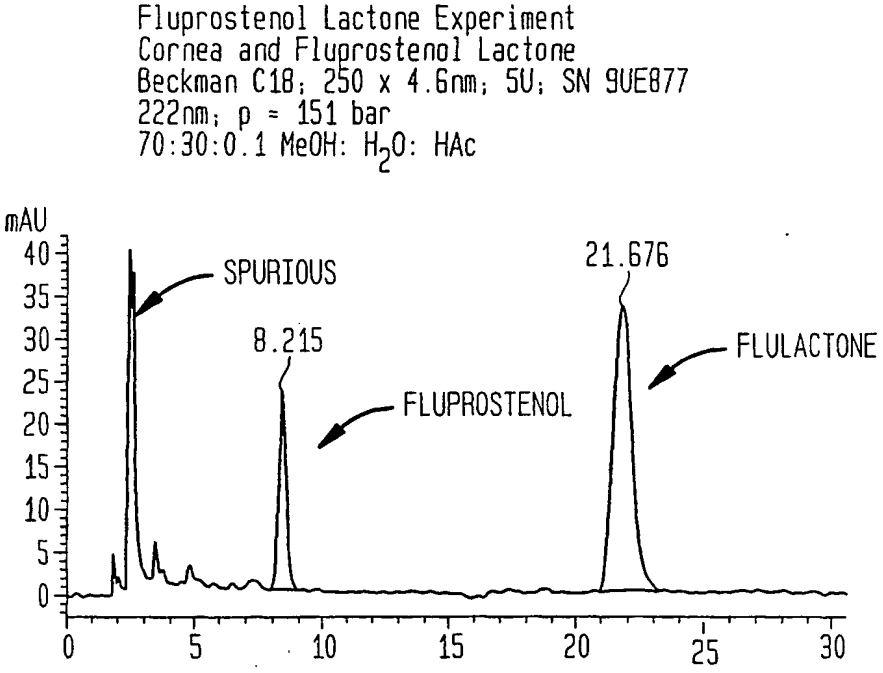


FIG. 4B



INTERNATIONAL SEARCH REPORT

Interna[redacted] Application No
PCT/US 01/03559

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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